EXHIBIT A

US008859562B2

(12) **United States Patent**
Helleday

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(45) **Date of Patent: Oct. 14, 2014**

(54) **USE OF RNAI INHIBITING PARP ACTIVITY FOR THE MANUFACTURE OF A MEDICAMENT FOR THE TREATMENT OF CANCER**

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(2), (4) Date: **Sep. 15, 2006**

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A61K 31/33	(2006.01)
A61K 31/435	(2006.01)
A61K 38/00	(2006.01)
C07D 487/06	(2006.01)
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A61K 31/5517	(2006.01)

(52) **U.S. Cl.**

CPC ***C07D 487/06*** (2013.01); ***A61K 38/005*** (2013.01); ***C12N 15/1137*** (2013.01); ***A61K 31/5517*** (2013.01); ***C12Y 204/0203*** (2013.01); *C12N 2310/14* (2013.01)

USPC **514/258.1**; 514/299; 514/388

(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

The present invention relates to the use of an agent that inhibits the activity of an enzyme that mediates repair of a DNA strand break in the manufacture of a medicament for the treatment of diseases caused by a defect in a gene that mediates homologous recombination.

1 Claim, 20 Drawing Sheets

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Figure 1.

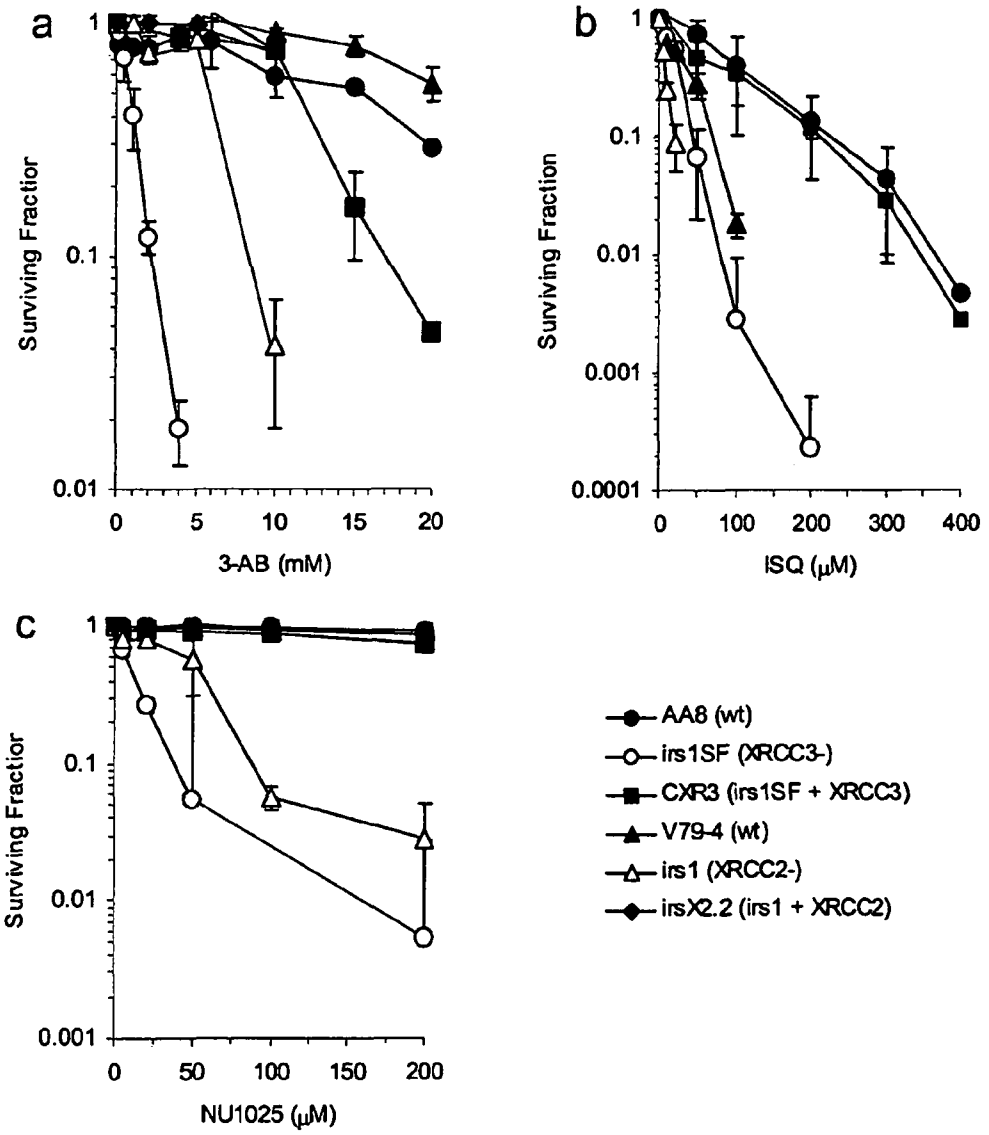

a
Surviving Fraction
1
0.1
0.01
0
5
10
15
20
3-AB (mM)
b
Surviving Fraction
1
0.1
0.01
0.001
0.0001
0
100
200
300
400
ISQ (μM)
c
Surviving Fraction
1
0.1
0.01
0.001
0
50
100
150
200
NU1025 (μM)
AA8 (wt)
irs1SF (XRCC3-)
CXR3 (irs1SF + XRCC3)
V79-4 (wt)
irs1 (XRCC2-)
irsX2.2 (irs1 + XRCC2)

Figure 2.

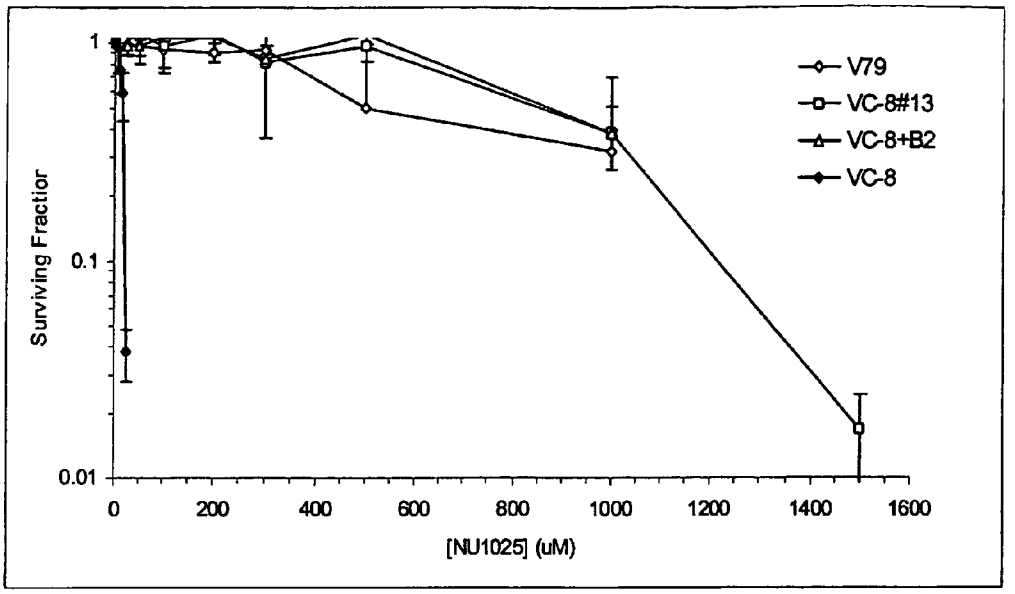

Surviving Fraction
1
0.1
0.01
0
200
400
600
800
1000
1200
1400
1600
[NU1025] (uM)
V79
VC-8#13
VC-8+B2
VC-8

Figure 3

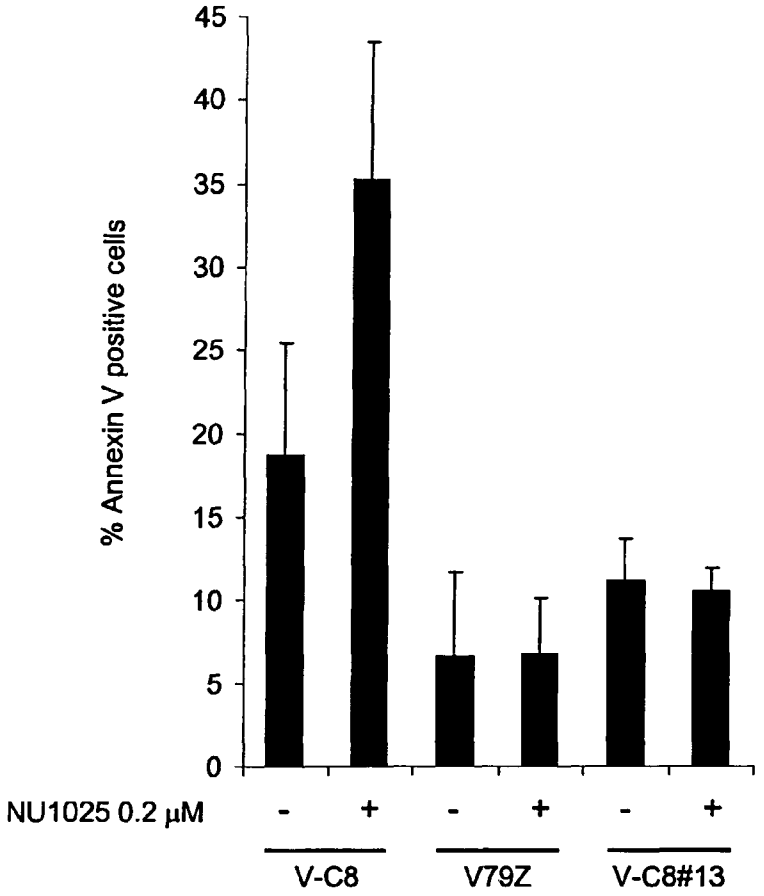

45
40
35
30
25
20
15
10
5
0
% Annexin V positive cells
NU1025 0.2 μM
-
+
-
+
-
+
V-C8
V79Z
V-C8#13

Figure 4

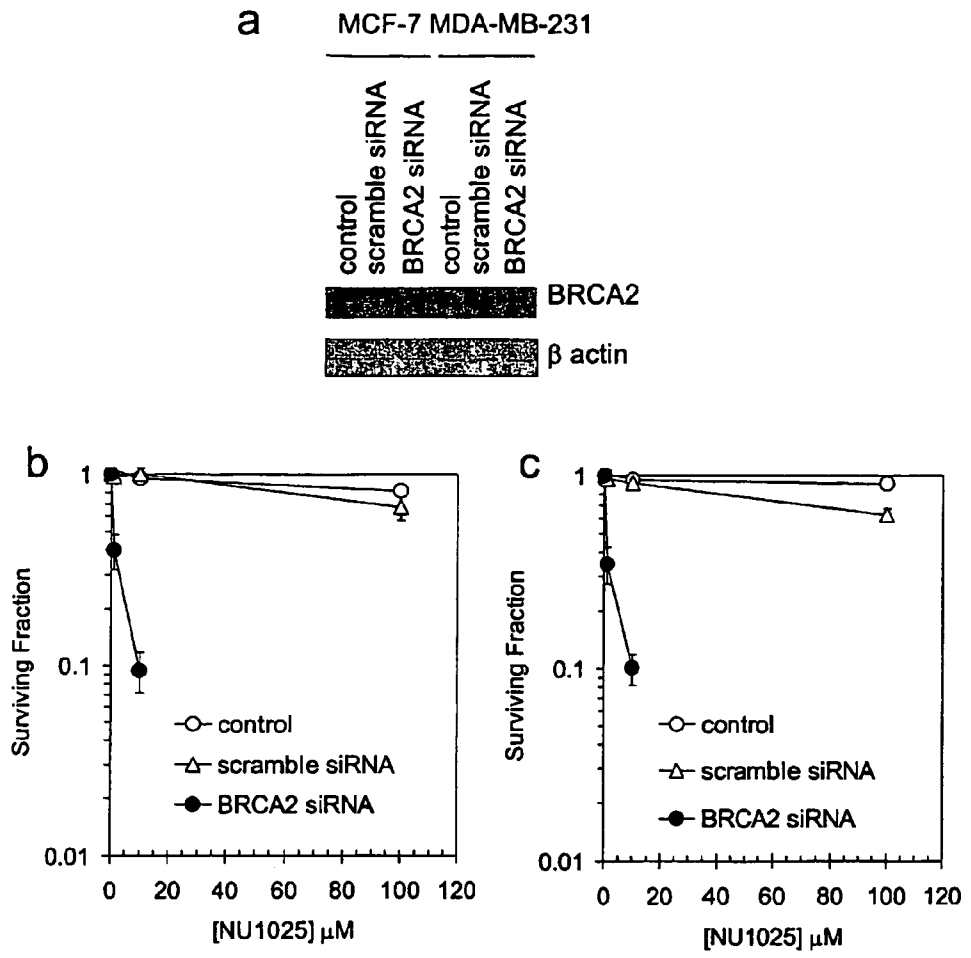
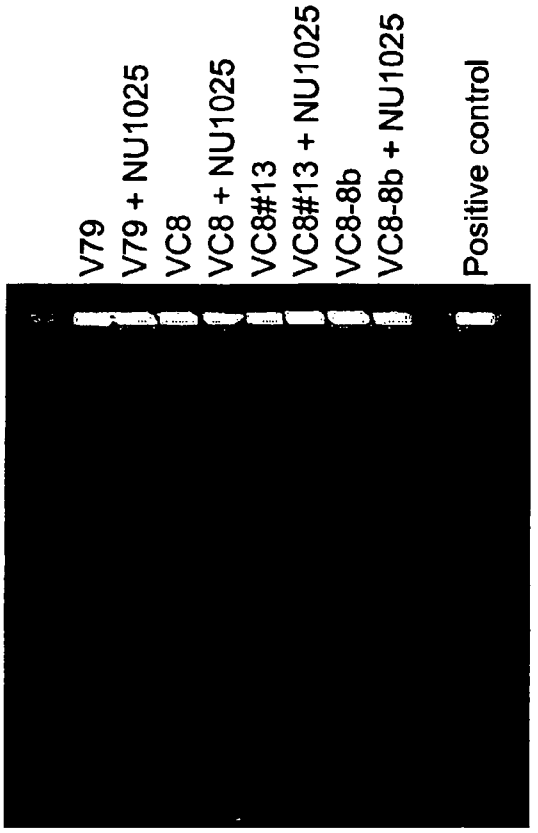

a
MCF-7 MDA-MB-231
control
scramble siRNA
BRCA2 siRNA
control
scramble siRNA
BRCA2 siRNA
BRCA2
β actin
b
Surviving Fraction
1
0.1
0.01
control
scramble siRNA
BRCA2 siRNA
0
20
40
60
80
100
120
[NU1025] μM
c
Surviving Fraction
1
0.1
0.01
control
scramble siRNA
BRCA2 siRNA
0
20
40
60
80
100
120
[NU1025] μM

Figure 5A


V79
V79 + NU1025
VC8
VC8 + NU1025
VC8#13
VC8#13 + NU1025
VC8-8b
VC8-8b + NU1025
Positive control

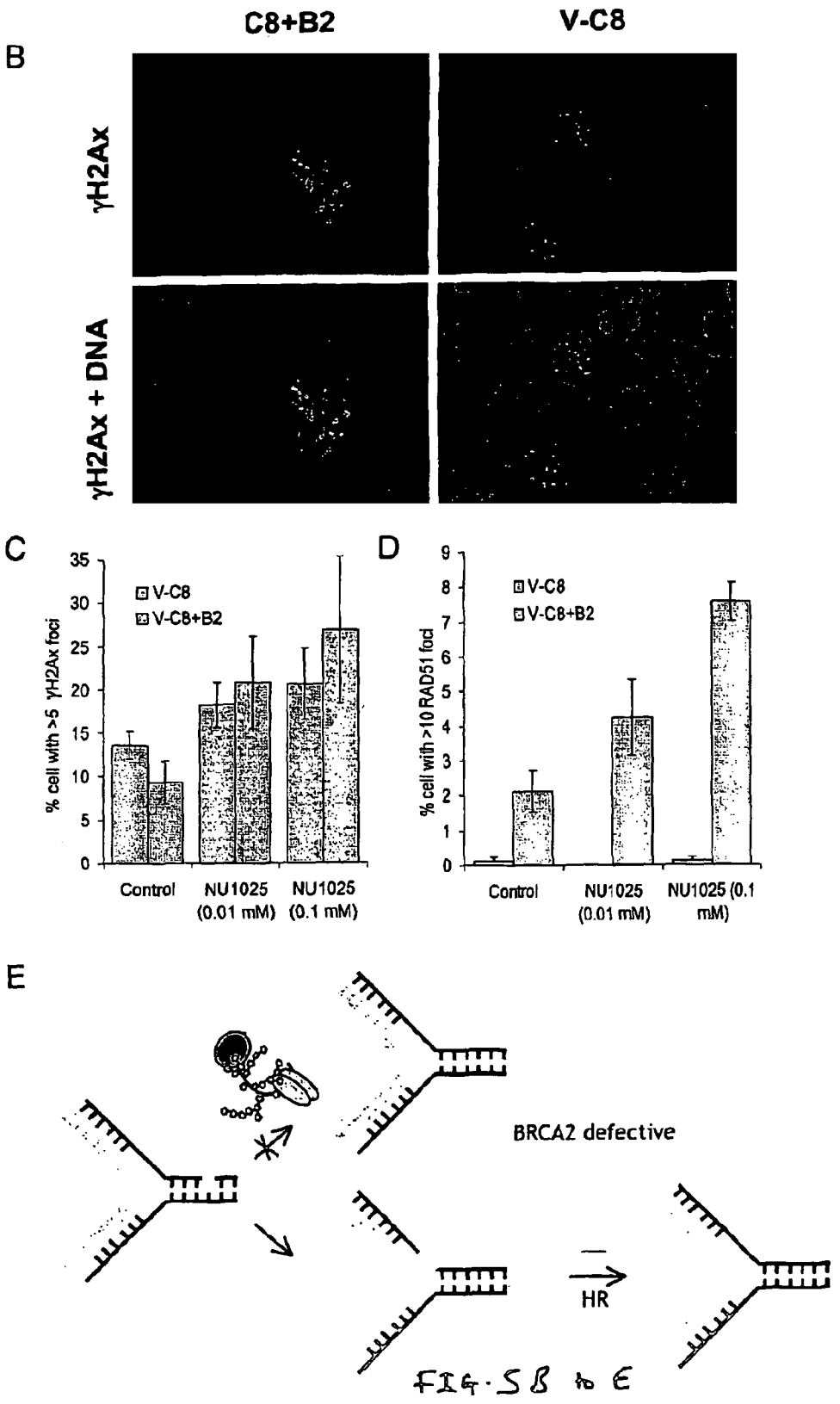

B
C8+B2
V-C8
γH2Ax
γH2Ax + DNA
C
% cell with >5 γH2Ax foci
V-C8
V-C8+B2
Control
NU1025 (0.01 mM)
NU1025 (0.1 mM)
D
% cell with >10 RAD51 foci
V-C8
V-C8+B2
Control
NU1025 (0.01 mM)
NU1025 (0.1 mM)
E
BRCA2 defective
HR

FIG. 5B to E

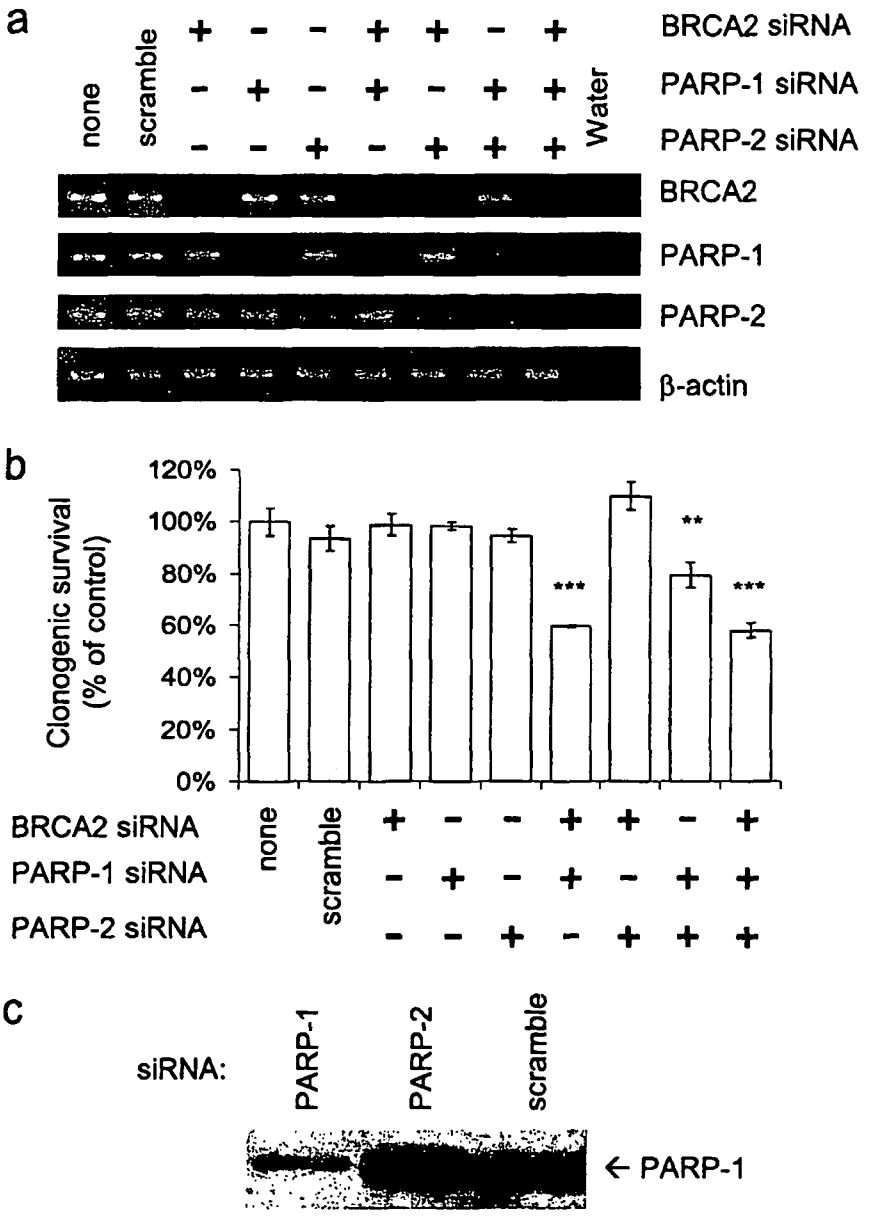

a
none
scramble
Water
BRCA2 siRNA
PARP-1 siRNA
PARP-2 siRNA
BRCA2
PARP-1
PARP-2
β-actin
b
Clonogenic survival (% of control)
120%
100%
80%
60%
40%
20%
0%
**
***
***
BRCA2 siRNA
PARP-1 siRNA
PARP-2 siRNA
none
scramble
c
siRNA:
PARP-1
PARP-2
scramble
← PARP-1

Fig. 6

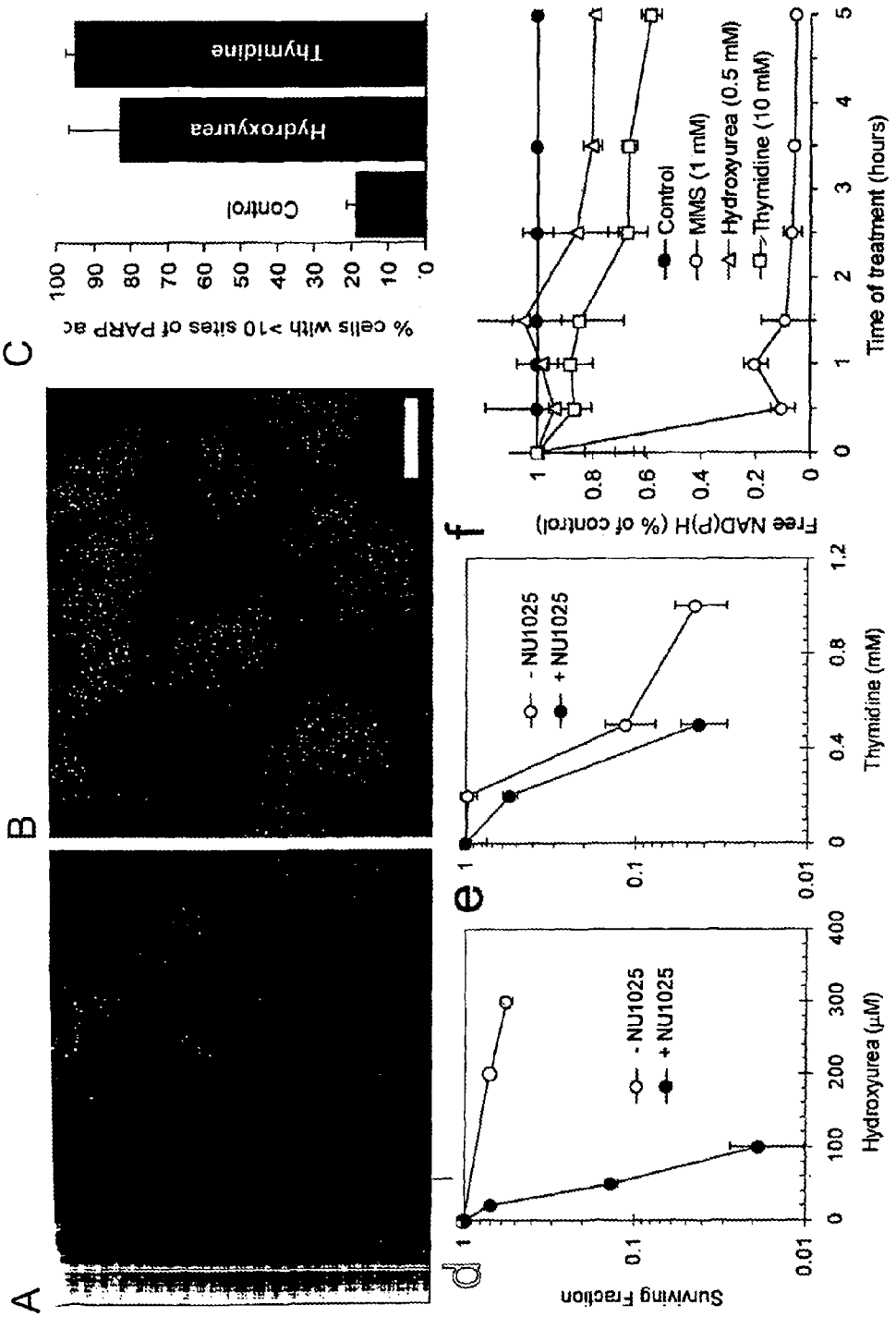

A
B
C
% cells with >10 sites of PARP ac
Control
Hydroxyurea
Thymidine
d
Surviving Fraction
- NU1025
+ NU1025
Hydroxyurea (μM)
e
- NU1025
+ NU1025
Thymidine (mM)
f
Free NAD(P)H (% of control)
Control
MMS (1 mM)
Hydroxyurea (0.5 mM)
Thymidine (10 mM)
Time of treatment (hours)

Fig. 7

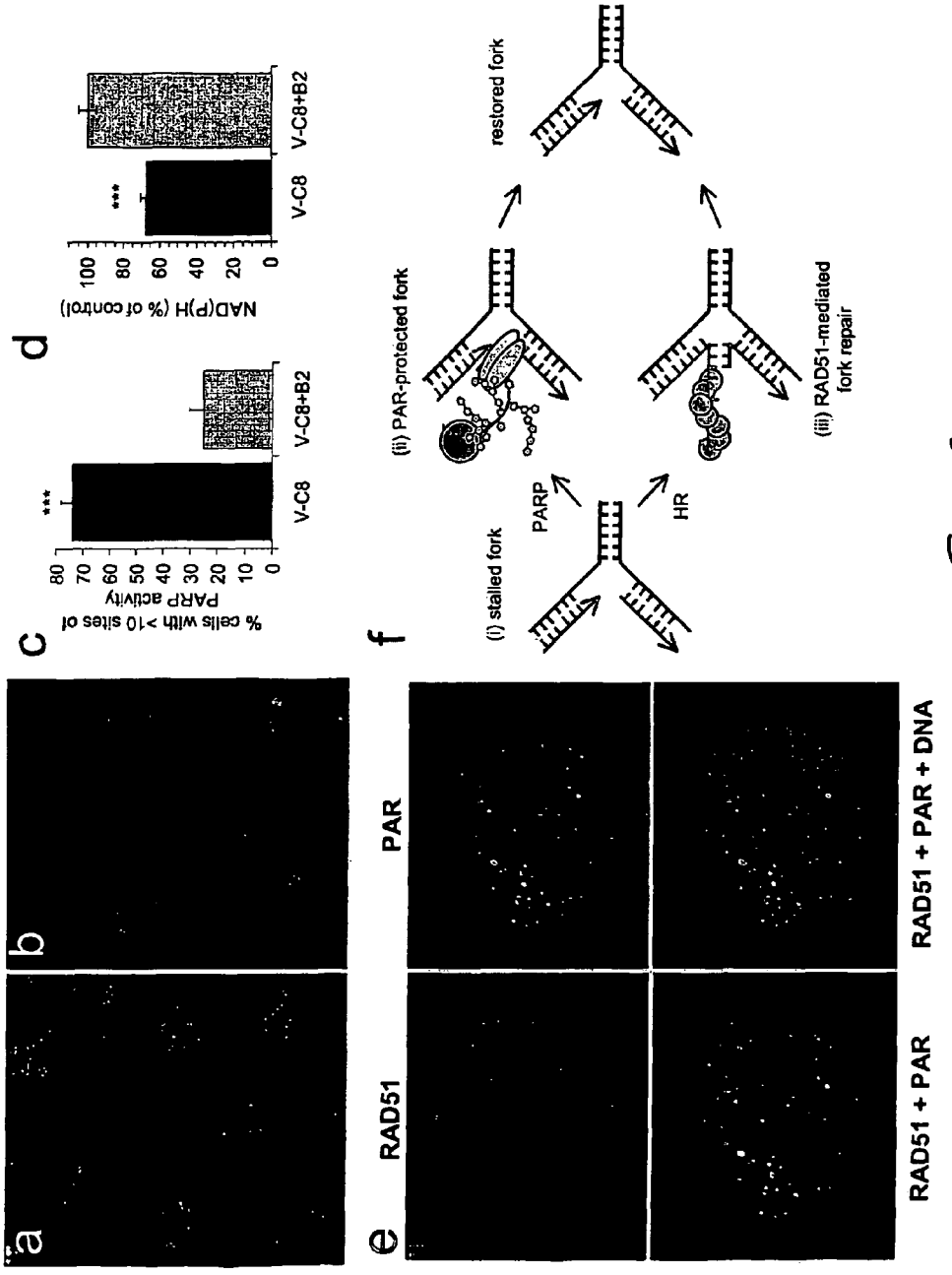

a
b
c
% cells with >10 sites of PARP activity
V-C8
V-C8+B2
***
d
NAD(P)H (% of control)
V-C8
V-C8+B2
***
e
RAD51
PAR
RAD51 + PAR
RAD51 + PAR + DNA
f
(i) stalled fork
PARP
HR
(ii) PAR-protected fork
restored fork
(iii) RAD51-mediated fork repair

FIG. 8

FIGURE 9

```
   1 cgcccgccca gccccggggg cagggaaagc ctaaattacg gaattaccgc gagcaaggag
  61 cgcggaatcg gggagcgtcc ggagctagct ggatcctcta ggcaggatgg tgatgggaat
 121 ctttgcaaat tgtatcttct gtttgaaagt gaagtactta cctcagcagc agaagaaaaa
 181 gctacaaact gacattaagg aaaatggcgg aaagttttcc ttttcgttaa atcctcagtg
 241 cacacatata atcttagata atgctgatgt tctgagtcag taccaactga attctatcca
 301 aaagaaccac gttcatattg caaacccaga ttttatatgg aaatctatca gagaaaagag
 361 actcttggat gtaaagaatt atgatcctta taagcccctg gacatcacac cacctcctga
 421 tcagaaggcg agcagttctg aagtgaaaac agaaggtcta tgcccggaca gtgccacaga
 481 ggaggaagac actgtggaac tcactgagtt tggtatgcag aatgttgaaa ttcctcatct
 541 tcctcaagat tttgaagttg caaaatataa caccttggag aaagtgggaa tggagggagg
 601 ccaggaagct gtggtggtgg agcttcagtg ttcgcgggac tccagggact gtcctttcct
 661 gatatcctca cacttcctcc tggatgatgg catggagact agaagacagt ttgctataaa
 721 gaaaacctct gaagatgcaa gtgaatactt tgaaaattac attgaagaac tgaagaaaca
 781 aggatttcta ctaagagaac atttcacacc tgaagcaacc caattagcat ctgaacaatt
 841 gcaagcattg cttttggagg aagtcatgaa ttcaagcact ctgagccaag aggtgagcga
 901 tttagtagag atgatttggg cagaggccct gggccacctg gaacacatgc ttctcaagcc
 961 agtgaacagg attagcctca acgatgtgag caaggcagag gggattctcc ttctagtaaa
1021 ggcagcactg aaaaatggag aaacagcaga gcaattgcaa aagatgatga cagagtttta
1081 cagactgata cctcacaaag gcacaatgcc caaagaagtg aacctgggac tattggctaa
1141 gaaagcagac ctctgccagc taataagaga catggttaat gtctgtgaaa ctaatttgtc
1201 caaacccaac ccaccatccc tggccaaata ccgagctttg aggtgcaaaa ttgagcatgt
1261 tgaacagaat actgaagaat ttctcagggt tagaaaagag gttttgcaga atcatcacag
1321 taagagccca gtggatgtct tgcagatatt tagagttggc agagtgaatg aaaccacaga
1381 gtttttgagc aaacttggta atgtgaggcc cttgttgcat ggttctcctg tacaaaacat
1441 cgtgggaatc ttgtgtcgag ggttgctttt acccaaagta gtggaagatc gtggtgtgca
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1561 tatcaagtac tcacacccgg gagagacaga tggcaccaga ctcctgctca tttgtgacgt
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1681 aggctacgac agtgtgcatg gagtttcaca aacagcctct gtcaccacag actttgagga
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2101 cgctgtgtgt ggcttcgaag ccttcatcaa tgggaagcac atagttggag agattaaaga
2161 gaaggaagaa gcccagcaag agtacctaga agccgtgacc cagggccatg gcgcttacct
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2281 taaggttctt ataaaaatta cctacatcac agaactcagc atcctgggca ctgttggtgt
2341 ctttttcatg cccgccaccg tagcaccctg gcaacaggac aaggctttga atgaaaacct
2401 tcaggataca gtagagaaga tttgtataaa agaaatagga acaaagcaaa gcttctcttt
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2521 gaaacaaaag cgcacagact gcaaagctgt cattagcacc atggaaggca gctccttaga
2581 cagcagtgga ttttctctcc acatcggttt gtctgctgcc tatctcccaa gaatgtgggt
2641 tgaaaaacat ccagaaaaag aaagcgaggc ttgcatgctt gtctttcaac ccgatctcga
2701 tgtcgacctc cctgacctag ccaatgagag cgaagtgatt atttgtcttg actgctccag
2761 ttccatggag ggtgtgacat tcttgcaagc caaggaaatc gccttgcatg cgctgtcctt
```

2821 ggtgggtgag aagcagaaag taaatattat ccagttcggc acaggttaca aggagctatt
2881 ttcgtatcct aagcatatca caagcaatac cgcggcagca gagttcatca tgtctgccac
2941 acctaccatg gggaacacag acttctggaa aacactccga tatcttagct tattgtaccc
3001 tgctcgaggg tcacggaaca tcctcctggt gtctgatggg cacctccagg atgagagcct
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3901 cttaggtgta ctaccagctt tcacatcaaa tttggaacgt ggacgtgtgg aaaagctatt
3961 ggatttaagt tggacagagt catgtaaacc aacagcaact gaaccactat ttaagaaagt
4021 cagtccatgg gaaacatcta cttctagctt ttttcctatt ttggctccgg ccgttggttc
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4201 ccaaggccct gtgcctggca cttgtgctga ctggatccca cagtcggcgt cttgtcccac
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5461 cttaaaat

Figure 9 (continued)

Figure 10

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Figure 11

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Figure 12

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Figure 12 (continued)

Figure 13

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3721 aatggcacat tctcctccag gtcatcactc agtcactggt aggcccagtg taaatggcct
3781 agcattagct gaatatgtta tttacagagg agaacaggct tatcctgagt atttaattac
3841 ttaccagatt atgaggcctg aaggtatggt cgatggataa atagttattt taagaaacta
3901 attccactga acctaaaatc atcaaagcag cagtggcctc tacgttttac tcctttgctg
3961 aaaaaaaatc atcttgccca caggcctgtg gcaaaaggat aaaaatgtga acgaagttta
4021 acattctgac ttgataaagc tttaataatg tacagtgttt tctaaatatt tcctgttttt
4081 tcagcacttt aacagatgcc attccaggtt aaactgggtt gtctgtacta aattataaac
4141 agagttaact tgaacctttt atatgttatg cattgattct aacaaactgt aatgccctca
4201 acagaactaa ttttactaat acaatactgt gttctttaaa acacagcatt tacactgaat
4261 acaatttcat ttgtaaaact gtaaataaga gcttttgtac tagcccagta tttatttaca
4321 ttgctttgta atataaatct gttttagaac tgcagcggtt tacaaaattt tttcatatgt
4381 attgttcatc tatacttcat cttacatcgt catgattgag tgatctttac atttgattcc
4441 agaggctatg ttcagttgtt agttgggaaa gattgagtta tcagatttaa tttgccgatg
4501 ggagccttta tctgtcatta gaaatctttc tcatttaaga acttatgaat atgctgaaga
4561 tttaatttgt gataccttg tatgtatgag acacattcca aagagctcta actatgatag
4621 gtcctgatta ctaaagaagc ttctttactg gcctcaattt ctagctttca tgttggaaaa
4681 ttttctgcag tccttctgtg aaaattagag caaagtgctc ctgtttttta gagaaactaa
4741 atcttgctgt tgaacaatta ttgtgttctt ttcatggaac ataagtagga tgttaacatt
4801 tccagggtgg gaagggtaat cctaaatcat ttcccaatct attctaatta ccttaaatct
4861 aaaggggaaa aaaaaaatca caaacaggac tgggtagttt tttatcctaa gtatattttt
4921 tcctgttctt tttacttggt tttattgctg tatttatagc caatctatac atcatgggta
4981 aacttaaccc agaactataa aatgtagttg tttcagtccc cttcaggcct cctgaatggg
5041 caagtgcagt gaaacaggtg cttcctgctc ctgggttttc tctccatgat gttatgccca
5101 attggaaata tgctgtcagt ttgtgcacca tatggtgacc acgcctgtgc tcagtttggc
5161 agctatagaa ggaaatgctg tcccataaaa tgccatccct atttctaata taacactctt
5221 ttccaggaag catgcttaag catcttgtta cagagacata catccattat ggcttggcaa
5281 tctcttttat ttgttgactc tagctccctt caaagtcgag gaaagatctt tactcactta
5341 atgaggacat tccccatcac tgtctgtacc agttcacctt tattttacgt tttattcagt
5401 ctgtaaatta actggccctt tgcagtaact tgtacataaa gtgctagaaa atcatgttcc
5461 ttgtcctgag taagagttaa tcagagtaag tgcatttctg gagttgtttc tgtgatgtaa
5521 attatgatca ttatttaaga agtcaaatcc tgatcttgaa gtgcttttta tacagctctc
5581 taataattac aaatatccga aagtcatttc ttggaacaca agtggagtat gccaaatttt
5641 atatgaattt ttcagattat ctaagcttcc aggttttata attagaagat aatgagagaa
5701 ttaatggggt ttatatttac attatctctc aactatgtag cccatattac tcaccctatg
5761 agtgaatctg gaattgcttt tcatgtgaaa tcattgtggt ctatgagttt acaatactgc

Figure 13 (continued)

5821 aaactgtgtt attttatcta aaccattgct taatgagtgt gtttttccat gaatgaatat
5881 accgtggttc atatgttagc atggcagcat tttcagatag ctttttgttt gttgggaagt
5941 tggggttttg gggggagggg gagtattagt acgttgcatg gaatagccta ctttataatg
6001 atgggaatgc ttttttcttt gttttgggat ttttttttt gaagtgaaat ttaacttttt
6061 gtgccagtag tactattata cccatcttca gtgtcttact tgtactgtat caaattccat
6121 accctcattt aattcttaat aaaactgttc acttgtaaaa aaaaaaaaaa aaaaaaaaaa
6181 aaaaaaaaa

Figure 13 (continued)

Figure 14

1 cgcccgccca gccccggggg cagggaaagc ctaaattacg gaattaccgc gagcaaggag
61 cgcggaatcg gggagcgtcc ggagctagct ggatcctcta ggcaggatgg tgatgggaat
121 ctttgcaaat tgtatcttct gtttgaaagt gaagtactta cctcagcagc agaagaaaaa
181 gctacaaact gacattaagg aaaatggcgg aaagttttcc ttttcgttaa atcctcagtg
241 cacacatata atcttagata atgctgatgt tctgagtcag taccaactga attctatcca
301 aaagaaccac gttcatattg caaacccaga ttttatatgg aaatctatca gagaaaagag
361 actcttggat gtaaagaatt atgatcctta taagcccctg gacatcacac cacctcctga
421 tcagaaggcg agcagttctg aagtgaaaac agaaggtcta tgcccggaca gtgccacaga
481 ggaggaagac actgtggaac tcactgagtt tggtatgcag aatgttgaaa ttcctcatct
541 tcctcaagat tttgaagttg caaaatataa caccttggag aaagtgggaa tggagggagg
601 ccaggaagct gtggtggtgg agcttcagtg ttcgcgggac tccagggact gtcctttcct
661 gatatcctca cacttcctcc tggatgatgg catggagact agaagacagt ttgctataaa
721 gaaaacctct gaagatgcaa gtgaatactt tgaaaattac attgaagaac tgaagaaaca
781 aggatttcta ctaagagaac atttcacacc tgaagcaacc caattagcat ctgaacaatt
841 gcaagcattg cttttggagg aagtcatgaa ttcaagcact ctgagccaag aggtgagcga
901 tttagtagag atgatttggg cagaggccct gggccacctg gaacacatgc ttctcaagcc
961 agtgaacagg attagcctca acgatgtgag caaggcagag gggattctcc ttctagtaaa
1021 ggcagcactg aaaaatggag aaacagcaga gcaattgcaa aagatgatga cagagtttta
1081 cagactgata cctcacaaag gcacaatgcc caaagaagtg aacctgggac tattggctaa
1141 gaaagcagac ctctgccagc taataagaga catggttaat gtctgtgaaa ctaatttgtc
1201 caaacccaac ccaccatccc tggccaaata ccgagctttg aggtgcaaaa ttgagcatgt
1261 tgaacagaat actgaagaat ttctcagggt tagaaaagag gttttgcaga atcatcacag
1321 taagagccca gtggatgtct tgcagatatt tagagttggc agagtgaatg aaaccacaga
1381 gtttttgagc aaacttggta atgtgaggcc cttgttgcat ggttctcctg tacaaaacat
1441 cgtgggaatc ttgtgtcgag ggttgctttt acccaaagta gtggaagatc gtggtgtgca
1501 aagaacagac gtcggaaacc ttggaagtgg gatttatttc agtgattcgc tcagtacaag
1561 tatcaagtac tcacacccgg gagagacaga tggcaccaga ctcctgctca tttgtgacgt
1621 agccctcgga aagtgtatgg acttacatga gaaggacttt cccttaactg aagcaccacc
1681 aggctacgac agtgtgcatg gagtttcaca aacagcctct gtcaccacag actttgagga
1741 tgatgaattt gttgtctata aaaccaatca ggttaaaatg aaatatatta ttaaattttc
1801 catgcctgga gatcagataa aggactttca tcctagtgat catactgaat tagaggaata
1861 cagacctgag ttttcaaatt tttcaaaggt tgaagattac cagttaccag atgccaaaac
1921 ttccagcagc accaaggccg gcctccagga tgcctctggg aacttggttc ctctggagga
1981 tgtccacatc aaagggagaa tcatagacac tgtagcccag gtcattgttt ttcagacata
2041 cacaaataaa agtcacgtgc ccattgaggc aaaatatatc tttcctttgg atgacaaggc
2101 cgctgtgtgt ggcttcgaag ccttcatcaa tgggaagcac atagttggag agattaaaga
2161 gaaggaagaa gcccagcaag agtacctaga agccgtgacc cagggccatg gcgcttacct
2221 gatgagtcag gatgctccgg acgtttttac tgtaagtgtt ggaaacttac cccctaaggc
2281 taaggttctt ataaaaatta cctacatcac agaactcagc atcctgggca ctgttggtgt
2341 ctttttcatg cccgccaccg tagcaccctg gcaacaggac aaggctttga atgaaaacct
2401 tcaggataca gtagagaaga tttgtataaa agaaatagga acaaagcaaa gcttctcttt
2461 gactatgtct attgagatgc cgtatgtgat tgaattcatt ttcagtgata cacatgaact
2521 gaaacaaaag cgcacagact gcaaagctgt cattagcacc atggaaggca gctccttaga
2581 cagcagtgga ttttctctcc acatcggttt gtctgctgcc tatctcccaa gaatgtgggt
2641 tgaaaaacat ccagaaaaag aaagcgaggc ttgcatgctt gtctttcaac ccgatctcga
2701 tgtcgacctc cctgacctag ccagtgagag cgaagtgatt atttgtcttg actgctccag

2761 ttccatggag ggtgtgacat tcttgcaagc caagcaaatc accttgcatg cgctgtcctt
2821 ggtgggtgag aagcagaaag taaatattat ccagttcggc acaggttaca aggagctatt
2881 ttcgtatcct aagcatatca caagcaatac cacggcagca gagttcatca tgtctgccac
2941 acctaccatg gggaacacag acttctggaa aacactccga tatcttagct tattgtaccc
3001 tgctcgaggg tcacggaaca tcctcctggt gtctgatggg cacctccagg atgagagcct
3061 gacattacag ctcgtgaaga ggagccgccc gcacaccagg ttattcgcct gcggtatcgg
3121 ttctacagca aatcgtcacg tcttaaggat tttgtcccag tgtggtgccg gagtatttga
3181 atattttaat gcaaaatcca agcatagttg gagaaaacag atagaagacc aaatgaccag
3241 gctatgttct ccgagttgcc actctgtctc cgtcaaatgg cagcaactca atccagatgc
3301 gcccgaggcc ctgcaggccc cagcccaggt gccatccttg tttcgcaatg atcgactcct
3361 tgtctatgga ttcattcctc actgcacaca agcaactctg tgtgcactaa ttcaagagaa
3421 agaattttgt acaatggtgt cgactactga gcttcagaag acaactggaa ctatgatcca
3481 caagctggca gcccgagctc taatcagaga ttatgaagat ggcattcttc acgaaaatga
3541 aaccagtcat gagatgaaaa aacaaacctt gaaatctctg attattaaac tcagtaaaga
3601 aaactctctc ataacacaat ttacaagctt tgtggcagtt gagaaaaggg atgagaatga
3661 gtcgcctttt cctgatattc caaaagtttc tgaacttatt gccaaagaag atgtagactt
3721 cctgccctac atgagctggc agggggagcc ccaagaagcc gtcaggaacc agtctctttt
3781 agcatcctct gagtggccag aattacgttt atccaaacga aaacatagga aaattccatt
3841 ttccaaaaga aaaatggaat tatctcagcc agaagtttct gaagattttg aagaggatgg
3901 cttaggtgta ctaccagctt tcacatcaaa tttggaacgt ggaggtgtgg aaaagctatt
3961 ggatttaagt tggacagagt catgtaaacc aacagcaact gaaccactat ttaagaaagt
4021 cagtccatgg gaaacatcta cttctagctt ttttcctatt ttggctccgg ccgttggttc
4081 ctatcttacc ccgactaccc gcgctcacag tcctgcttcc ttgtcttttg cctcatatcg
4141 tcaggtagct agtttcggtt cagctgctcc tcccagacag tttgatgcat ctcaattcag
4201 ccaaggccct gtgcctggca cttgtgctga ctggatccca cagtcggcgt cttgtcccac
4261 aggacctccc cagaacccac cttctgcacc ctattgtggc attgtttttt cagggagctc
4321 attaagctct gcacagtctg ctccactgca acatcctgga ggctttacta ccaggccttc
4381 tgctggcacc ttccctgagc tggattctcc ccagcttcat ttctctcttc ctacagaccc
4441 tgatcccatc agaggttttg ggtcttatca tccctctgct tactctcctt ttcattttca
4501 accttccgca gcctctttga ctgccaacct taggctgcca atggcctctg ctttacctga
4561 ggctctttgc agtcagtccc ggactacccc agtagatctc tgtcttctag aagaatcagt
4621 aggcagtctc gaaggaagtc gatgtcctgt ctttgctttt caaagttctg acacagaaag
4681 tgatgagcta tcagaagtac ttcaagacag ctgcttttta caaataaagt gtgatacaaa
4741 agatgacagt atcccgtgct ttctggaatt aaaagaagag gatgaaatag tgtgcacaca
4801 acactggcag gatgctgtgc cttggacaga actcctcagt ctacagacag aggatggctt
4861 ctggaaactt acaccagaac tgggacttat attaaatctt aatacaaatg gtttgcacag
4921 ctttcttaaa caaaaaggca ttcaatctct aggtgtaaaa ggaagagaat gtctcctgga
4981 cctaattgcc acaatgctgg tactacagtt tattcgcacc aggttggaaa aagagggaat
5041 agtgttcaaa tcactgatga aaatggatga cccttctatt tccaggaata ttccctgggc
5101 ttttgaggca ataaagcaag caagtgaatg ggtaagaaga actgaaggac agtacccatc
5161 tatctgccca cggcttgaac tggggaacga ctgggactct gccaccaagc agttgctggg
5221 actccagccc ataagcactg tgtcccctct tcatagagtc ctccattaca gtcaaggcta
5281 agtcaaatga aactgaattt taaacttttt gcatgcttct atgtagaaaa taatcaaatg
5341 ataatagata attataatga aacttcatta aggtttcatt cagtgtagca attactgtct
5401 ttaaaaatta agtggaagaa gaattacttt aatcaactaa caagcaataa taaaatgaaa
5461 cttaaaataa aaaaaaaaaa aaaaaaaaaa

Figure 14 (continued)

USE OF RNAI INHIBITING PARP ACTIVITY FOR THE MANUFACTURE OF A MEDICAMENT FOR THE TREATMENT OF CANCER

This invention relates to the use of an agent that inhibits the activity of an enzyme which mediates the repair of DNA strand breaks in the treatment of certain forms of cancer in particular breast cancer.

Homologous recombination (HR) has been shown to play an important role in repair of damage occurring at DNA replication forks in mammalian cells (2). Thus, cells deficient in HR show retarded growth and exhibit higher level of genetic instability. It is believed that genetic instability due to loss of HR repair in human cancers significantly contributes to the development of cancer in these cells (1).

Post transcriptional modification of nuclear proteins by poly(ADP-ribosyl)ation (PARP) in response to DNA strand breaks plays an important role in DNA repair, regulation of apoptosis, and maintenance of genomic stability.

Poly(ADP-ribose)Polymerase (PARP-1) is an abundant nuclear protein in mammalian cells that catalyses the formation of poly(ADP-ribose) (PAR) polymers using NAD^+ as substrate. Upon DNA damage, PARP-1 binds rapidly to a DNA strand break (single strand or double strand) and catalyses the addition of negatively charged PAR chains to itself (automodification) and other proteins (see [3, 4] for reviews). The binding of PARP-1 to DNA strand breaks is believed to protect DNA lesions from further processing until PARP-1 is dissociated from the break by the accumulated negative charge resulting from PAR polymers (5,6).

Although PARP-1 has been implicated in several nuclear processes, such as modulation of chromatin structure, DNA replication, DNA repair and transcription, PARP-1 knockout mice develop normally (7). Cells isolated from these mice exhibit a hyper recombination phenotype and genetic instability in the form of increased levels of SCE, micronuclei and tetraploidy (8-10). Genetic instability may also occur in these PARP-1 knockout mice through telomere shortening, increased frequency of chromosome fusion and aneuploidy (11), although all of these results could not be repeated in another set of PARP-1 knock-out mice (12). In the former mice knockout, PARP-1 null mutation rescue impaired V(D)J recombination in SCID mice (13).

These results support the view suggested by Lindahl and coworkers that PARP-1 has a protective role against recombination (5). They proposed that binding of PARP-1 to DNA strand breaks prevents the recombination machinery from recognizing and processing DNA lesions or, alternatively, that the negative charges accumulated following poly ADP-ribosylation repel adjacent recombinogenic DNA sequences. Only the latter model is consistent with inhibition of PARP-1 itself and expression of a dominant negative mutant PARP-1, inducing SCE, gene amplification and homologous recombination (HR [14-18]).

Studies based on treating cells with PARP inhibitors or cells derived from PARP-1 or PARP-2 knockout mice indicate that the suppression of PARP-1 activity increases cell susceptibility to DNA damaging agents and inhibits strand break rejoining (3, 4, 8-11, 19, 20, 47).

Inhibitors of PARP-1 activity have been used in combination with traditional anti-cancer agents such as radio therapy and chemotherapy (21). The inhibitors were used in combination with methylating agents, topoisomerase poisons and ionising radiations and were found to enhance the effectiveness of these forms of treatment. Such treatments, however, are known to cause damage and death to non cancerous or "healthy" cells and are associated with unpleasant side effects.

There is therefore a need for a treatment for cancer that is both effective and selective in the killing of cancer cells and which does not need to be administered in combination with radio or chemotherapy treatments.

The present inventors have surprisingly found that cells deficient in homologous recombination (HR) are hypersensitive to PARP inhibitors as compared to wild type cells. This is surprising since PARP-1 knockout mice live normally thereby indicating that PARP-1 is not essential for life. Thus, it could not be expected that cells would be sensitive to PARP inhibition.

According to a first aspect of the invention there is provided the use of an agent that inhibits the activity of an enzyme that mediates the repair of DNA strand breaks in the manufacture of a medicament for the treatment of diseases that are caused by a genetic defect in a gene that mediates homologous recombination.

In a further aspect the invention provides a method of treatment of a disease or condition in a mammal, including human, which is caused by a genetic defect in a gene which mediates homologous recombination, which method comprises administering to the mammal a therapeutically effective amount of an agent which inhibits the activity of an enzyme which mediates repair of DNA strand breaks or other lesions present at replication forks.

In a preferred aspect said enzyme is PARP. In a further preferred aspect said agent is a PARP inhibitor or an RNAi molecule specific to PARP gene.

In a further preferred aspect, the use is in the treatment of cancer.

Preferably the medicament is a pharmaceutical composition consisting of the PARP inhibitor in combination with a pharmaceutically acceptable carrier or diluent.

The specific sensitivity of HR defective tumours to PARP-1 inhibition means that normally dividing cells in the patient will be unaffected by the treatment. Treatment of HR defective cancer cells using a PARP inhibitor also has the advantage that it does not need to be administered as a combination therapy along with conventional radio or chemotherapy treatments thereby avoiding the side effects associated with these conventional forms of treatment.

A genetic defect in a gene which mediates homologous recombination may be due to a mutation in, the absence of, or defective expression of, a gene encoding a protein involved in HR.

In a further aspect, the invention further provides the use of a PARP inhibitor in the manufacture of a medicament for inducing apoptosis in HR defective cells.

In another aspect the invention provides a method of inducing apoptosis in HR defective cells in a mammal which method comprises administering to the mammal a therapeutically effective amount of a PARP inhibitor.

By causing apoptosis in HR defective cells it should be possible to reduce or halt the growth of a tumour in the mammal.

Preferably, the HR defective cells are cancer cells.

Cancer cells defective in HR may partially or totally deficient in HR. Preferably the cancer cells are totally deficient in HR.

The term "cancer" or "tumour" includes lung, colon, pancreatic, gastric, ovarian, cervical, breast or prostate cancer. The cancer may also include skin, renal, liver, bladder or cerebral cancer. In a preferred aspect, the cancer is in a mammal, preferably human.

The cancer to be treated may be an inherited form of cancer wherein the patient to be treated has a familial predisposition to the cancer. Preferably, the cancer to be treated is gene-linked hereditary cancer. In a preferred embodiment of the invention the cancer is gene-linked hereditary breast cancer.

In a preferred aspect, the PARP inhibitor is useful in the treatment of cancer cells defective in the expression of a gene involved in HR. Genes with suggested function in HR include XRCC1, ADPRT (PARP-1), ADPRTL2 (PARP-2), CTPS, RPA, RPA1, RPA2, RPA3, XPD, ERCC1, XPF, MMS19, RAD51, RAD51B, RAD51C, RAD51D, DMC1, XRCC2, XRCC3, BRCA1, BRCA2, RAD52, RAD54, RAD50, MRE11, NBS1, WRN, BLM, Ku70, Ku80, ATM, ATR, chk1, chk2, FANCA, FANCB, FANCC, FANCD1, FANCD2, FANCE, FANCF, FANCG, RAD1, RAD9, FEN-1, Mus81. Eme1, DDS1, BARD (see (2, 3, 5, 22-28) for reviews).

A gene involved in HR may be a tumour suppressor gene. The invention thus provides for the treatment of cancer cells defective in the expression of a tumour suppressor gene. Preferably, the tumour suppressor gene is BRCA1 or BRCA2.

Breast cancer is the most common cancer disease among women in the Western world today. Certain families have strong predisposition for breast cancer, which is often owing to an inherited mutation in one allele of either BRCA1 or BRCA2. However, these patients still maintain one functional allele. Thus, these patients develop normally and have no phenotypic consequence from this mutation. However, in one cell, the functional allele might be lost, making this cell cancerous and at the same time deficient in homologous recombination (HR). This step is critical for the onset of a tumour (1).

The present inventors have surprisingly found that BRCA2 deficient cells are 100 times more sensitive to the cytotoxicity of the PARP inhibitor, NU1025, than wild type cells.

Thus in a preferred aspect, the invention provides the use of a PARP inhibitor in the manufacture of a medicament for the treatment of cancer cells defective in HR, e.g due to the loss of BRCA1 and/or BRCA2 expression.

The cancer cells to be treated may be partially or totally deficient in BRCA1 or BRCA2 expression. BRCA1 and BRCA2 mutations can be identified using multiplex PCR techniques, array techniques (29, 30) or using other screens known to the skilled person.

PARP inhibitors useful in the present invention may be selected from inhibitors of PARP-1, PARP-2, PARP-3, PARP-4, tankyrase 1 or tankyrase 2 (see 31 for a review). In a preferred embodiment, the PARP inhibitor useful in the present invention is an inhibitor of PARP-1 activity.

PARP inhibitors useful in the present invention include benzimidazole-carboxamides, quinazolin-4-[3H]-ones and isoquinoline derivatives (e.g. 2-(4-hydroxyphenyl)benzimidazole-4-carboxamide (NU1085), 8-hydroxy-2-methylquinazolin-4-[3H]one (NU1025); 6(5H)phenanthridinone; 3 aminobenzamide; benzimidazole-4-carboxamides (BZ1-6) and tricyclic lactam indoles (TI1-5) [32]. Further inhibitors of PARP may be identified either by design [33] or the novel FlashPlate assay [34].

The PARP inhibitor formulated as a pharmaceutical composition may be administered in any effective, convenient manner effective for targeting cancer cells including, for instance, administration by oral, intravenous, intramuscular, intradermal, intranasal, topical routes among others. Carriers or diluents useful in the pharmaceutical composition may include, but are not limited to saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof.

In therapy or as a prophylactic, the active agent may be administered to an individual as an injectable composition, for example as a sterile aqueous dispersion. The inhibitor may be administered directly to a tumour or may be targeted to the tumour via systemic administration.

A therapeutically effective amount of the inhibitor is typically one which is sufficient to achieve the desired effect and may vary according to the nature and severity of the disease condition, and the potency of the inhibitor. It will be appreciated that different concentrations may be employed for prophylaxis than for treatment of an active disease.

For administration to mammals, and particularly humans, it is expected that the daily dosage level of the active agent will be up to 100 mg/kg, for example from 0.01 mg/kg to 50 mg/kg body weight, typically up to 0.1, 0.5, 1.0, 2.0 5.0, 10, 15, 20 or 30 mg/kg body weight. Ultimately, however, the amount of inhibitor administered and the frequency of administration will be at the discretion of a physician.

A therapeutic advantage of using PARP inhibitors to treat cancer cells is that only very low doses are needed to have a therapeutic effect in treating cancer thereby reducing systemic build up of the inhibitors and any associated toxic effects.

A preferred aspect of the invention provides an agent which is an inhibitory RNA (RNAi) molecule.

A technique to specifically ablate gene function is through the introduction of double stranded RNA, also referred to as inhibitory RNA (RNAi), into a cell which results in the destruction of mRNA complementary to the sequence included in the RNAi molecule. The RNAi molecule comprises two complementary strands of RNA (a sense strand and an antisense strand) annealed to each other to form a double stranded RNA molecule. The RNAi molecule is typically derived from exonic or coding sequence of the gene which is to be ablated.

Preferably said RNAi molecule is derived from the nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of:

a) a nucleic acid sequence as represented by the sequence in FIG. **9**, **10**, **11**, **12**, **13** or **14** or fragment thereof;

b) a nucleic acid sequence which hybridises to the nucleic acid sequences of FIG. **9**, **10**, **11**, **12**, **13** or **14** and encodes a gene for PARP;

c) a nucleic acid sequence which comprise sequences which are degenerate as a result of the genetic code to the nucleic acid sequences defined in (a) and (b).

Recent studies suggest that RNAi molecules ranging from 100-1000 bp derived from coding sequence are effective inhibitors of gene expression. Surprisingly, only a few molecules of RNAi are required to block gene expression which implies the mechanism is catalytic. The site of action appears to be nuclear as little if any RNAi is detectable in the cytoplasm of cells indicating that RNAi exerts its effect during mRNA synthesis or processing.

More preferably said RNAi molecule according has a length of between 10 nucleotide bases (nb)-1000 nb. Even more preferably said RNAi molecule has a length of 10 nb; 20 nb; 30 nb; 40 nb; 50 nb; 60 nb; 70 nb; 80 nb; 90 nb; or 100 bp. Even more preferably still said RNAi molecule is 21 nb in length.

Even more preferably still the RNAi molecule comprises the nucleic acid sequence aaa agc cau ggu gga gua uga (PARP-1)

Even more preferably still the RNAi molecule consists of the nucleic acid sequence aag acc aau cuc ucc agu uca ac (PARP-2)

Even more preferably still the RNAi molecule consists of the nucleic acid sequence aag acc aac auc gag aac aac (PARP-3)

The RNAi molecule may comprise modified nucleotide bases.

Preferred features of each aspect of the invention are as for each of the other aspects *mutatis mutandis*.

The present invention will now be described by way of example only with reference to the accompanying figures, wherein:

FIG. **1** is a graph demonstrating that HR deficient cells are hypersensitive to the toxic effect caused by inhibition of PARP-1. Colony outgrowth of the Chinese hamster cell lines AA8 (wild-type), irs1SF (deficient in HR[4]), CXR3 (irs1SF complemented with XRCC3 [2]), V79 (wild-type), irs1 (deficient in HR[5]) or irs1X2.2 (irs1 complimented with XRCC2 [1]) upon exposure to 3-AB (A), ISQ (B) or NU1025 (C). The means (symbols) and standard deviation (bars) of at least three experiments are shown. Colony outgrowth assay was used;

FIG. **2** is a graph showing cell survival in the presence of PARP inhibitor NU1025 in wt V79 cells, BRCA2 deficient VC-8 cells and VC-8 cells complimented with functional BRCA2 gene (VC-8#13, VC-8+B2). Colony outgrowth assay was used;

FIG. **3** is a histogram showing the percentage of the cells in apoptosis following a 72 hour incubation with NU1025;

FIG. **4**. (a) Western blot analysis of protein lysates isolated from MCF-7 ($p53^{wt}$) or MDA-MB-231 ($p53^{mut}$) breast cancer cells following 48 hours transfection with siRNA. (b) Colony outgrowth of siRNA-treated MCF-7 cells or (c) MDA-MB-231 cells following exposure to the PARP inhibitor NU1025. The means (symbols) and standard deviation (bars) of at least three experiments are shown.

FIG. **5**. BRCA2 deficient cells fail to repair a recombination lesion formed at replication forks by inhibitors of PARP. (a) Visualization of double strand breaks (DSBs) in BRCA2 proficient or deficient cells following a 24-hour treatment with NU1025 (0.1 mM) by pulse-field gel electrophoresis. Hydroxyurea 2 mM was used as a positive control. (b) Visualisation of γH2Ax foci in untreated V-C8+B2 and V-C8 cells. Number of cells containing γH2Ax foci (c) or RAD51 foci (d) visualised in V-C8+B2 and V-C8 cells following a 24-hour treatment with NU1025 (10 μM). The means (symbols) and standard errors (bars) of three to nine experiments are shown. (e) A suggested model for cell death induced in BRCA2 deficient cells.

FIG. **6**. PARP-1 and not PARP-2 is important in preventing formation of a recombinogenic lesion, causing death in absence of BRCA2. (a) RT-PCR on RNA isolated from SW480SN.3 cells treated with BRCA2, PARP-1 and PARP-2 siRNA in combinations as shown for 48 hours. (b) Clonogenic survival following 48-hours depletion of BRCA2, PARP-1 and PARP-2. The means (symbols) and standard deviation (bars) of at least three experiments are shown. Two and three stars designate statistical significance in t-test $p<0.01$ and $p<0.001$, respectively. (c) Western blot for PARP-1 in SW480SN.3 cells treated with different siRNA.

FIG. **7**. (a) Visualisation of PAR polymers in untreated and (b) thymidine treated V79 cells (5 mM for 24 hours). (c) Percentage cells containing >10 sites of PARP activity following treatment with hydroxyurea (0.2 mM) and thymidine (5 mM). At least 300 nuclei were counted for each treatment and experiment. (d) Survival of V-C8+B2 cells following co-treatment with hydroxyurea or (e) thymidine and NU1025 (10 μM). (f) The activity of PARP was measured by the level of free $NAD(P)H^{11}$, following treatment with MMS, hydroxyurea (0.5 mM) or thymidine (10 mM). The means (symbol) and standard deviation (error bars) from at least three experiments are depicted.

FIG. **8**. (a) Visualisation of PAR polymers in untreated V-C8 and (b) V-C8+B2 cells. (c) Quantification of percentage cells containing >10 sites of PARP activity in untreated V-C8 and V-C8+B2 cells. (d) Level of NAD(P)H measured in untreated V-C8 and V-C8+B2 cells. Three stars designate $p<0.001$ in t-test. (e) Visualization of RAD51 and sites of PARP activity in V79 cells following a 24-hour thymidine treatment (5 mM). (f) A model for the role of PARP and HR at stalled replication forks.

FIG. **9** is the human cDNA sequence of PARP-1;

FIG. **10** is the human cDNA sequence of PARP-2;

FIG. **11** is the human cDNA sequence of PARP-3;

FIG. **12** is the human gDNA sequence of Tankyrase 1;

FIG. **13** is the human mRNA sequence of Tankyrase 2;

FIG. **14** is the human mRNA sequence of VPARP.

MATERIALS AND METHODS

Cytotoxicity of PARP Inhibitors to HR-Defective Cells: XRCC2, XRCC3 or BRCA2

Cell Culture

The irs1, irs1X2.1 and V79-4 cell lines were a donation from John Thacker [40] and the AA8, irs1SF and CXR3 cell lines were provided by Larry Thompson [41].

The VC-8, VC-8+B2, VC-8#13 were a gift from Malgorzata Zdzienicka [42]. All cell lines in this study were grown in Dulbecco's modified Eagle's Medium (DMEM) with 10% Foetal bovine serum and penicillin (100 U/ml) and streptomycin sulphate (100 μg/mL) at 37° C. under an atmosphere containing 5% CO_2.

Toxicity Assay—Colony Outgrowth Assay

500 cells suspended in medium were plated onto a Petri dish 4 hours prior to the addition of 3-AB, ISQ or NU1025. ISQ and NU1025 were dissolved in DMSO to a final concentration of 0.2% in treatment medium. 7-12 days later, when colonies could be observed, these colonies were fixed and stained with methylene blue in methanol (4 g/l). Colonies consisting of more than 50 cells were subsequently counted.

Apoptosis Experiments

0.25×10^6 cells were plated onto Petri dishes and grown for 4 hours before treatment with NU1025. After 72 hours, cells were trypsinized and resuspended with medium containing any floating cells from that sample. The cells were pelleted by centrifugation and resuspended for apoptosis analysis with FITC-conjugated annexin-V and propidium iodine (PI) (ApoTarget, Biosource International) according to manufacturer's protocol. Samples were analysed by flow cytometry (Becton-Dickenson FACSort, 488 nm laser), and percentage of apoptotic cells was determined by the fraction of live cells (PI-negative) bound with FITC-conjugated annexin-V.

Immunofluorescence

Cells were plated onto coverslips 4 h prior to 24-h treatments as indicated. Following treatments the medium was removed and coverslips rinsed once in PBS at 37° C. and fixed as described elsewhere [2]. The primary antibodies and dilutions used in this study were; rabbit polyclonal anti PAR (Trevigen; 1:500), goat polyclonal anti Rad51 (C-20, Santa Cruz; 1:200) and rabbit polyclonal anti Rad51 (H-92, Santa Cruz; 1:1000). The secondary antibodies were Cy-3-conjugated goat anti-rabbit IgG antibody (Zymed; 1:500), Alexa 555 goat anti-rabbit $F(ab')_2$IgG antibody (Molecular Probes; 1:500), Alexa 546 donkey anti-goat IgG antibody (Molecular Probes; 1:500) and Alexa 488 donkey anti-rabbit IgG antibody (Molecular Probes; 1:500). Antibodies were diluted in

PBS containing 3% bovine serum albumin. DNA was stained with 1 μg/ml To Pro (Molecular Probes). Images were obtained with a Zeiss LSM 510 inverted confocal microscope using planapochromat 63X/NA 1.4 oil immersion objective and excitation wavelengths 488, 546 and 630 nm. Through focus maximum projection images were acquired from optical sections 0.50 μm apart and with a section thickness of 1.0 μm. Images were processed using Adobe PhotoShop (Abacus Inc). At least 300 nuclei were counted on each slide and those containing more than 10 RAD51 foci or sites of PARP activity were classified as positive.

PARP Activity Assays

A water-soluble tetrazolium salt (5 mM WST-8) was used to monitor the amount of NAD(P)H through its reduction to a yellow coloured formazan dye[43]. 5000 cells were plated in at least triplicate into wells of a 96 well plate and cultured in 100 μl normal growth media for 4 h at 37° C. CK8 buffer (Dojindo Molecular Technology, Gaithersburg, USA), containing WST-8, was then added either with or without treatment with DNA damaging agents at concentrations indicated. Reduction of WST-8 in the presence of NAD(P)H was determined by measuring visible absorbance (OD_{450}) every 30 min. A medium blank was also prepared containing just media and CK8 buffer. Changes in NAD(P)H levels were calculated by comparing the absorbance of wells containing cells treated with DNA damaging agents and those treated with DMSO alone. Alternately relative levels of NAD(P)H in different cells lines were calculated after 4 h incubation in CK8 buffer.

The ability of NU1025 to inhibit PARP-1 activity was also assayed in permeabilised cells using a modification of the method of Halldorsson et al [44], and described in detail elsewhere [45]. Briefly: 300 μl of NU1025-treated (15 min) permeabilised cells were incubated at 26° C. with oligonucleotide (final conc. 2.5 μg/ml), 75 μM NAD+[^{32}P] NAD (Amersham Pharmacia, Amersham, UK) in a total volume of 400 μl. The reaction was terminated after 5 min by adding ice cold 10% TCA 10% Na Ppi for 60 min prior to filtering through a Whatman GF/C filter (LabSales, Maidstone, UK), rinsed 6× with 1% TCA 1% NaPPi, left to dry and incorporated radioactivity was measured to determine PARP-1 activity. Data are expressed as pmol NAD incorporated/10^6 cells by reference to [^{32}P] NAD standards.

Pulse-Field Gel Electrophoresis

1.5×10^6 cells were plated onto 100 mm dishes and allowed 4 h for attachment. Exposure to drug was for 18 h after which cells were trypsinsied and 10^6 cells melted into each 1% agarose insert. These inserts were incubated as described elsewhere (8) and separated by pulse-field gel electrophoresis for 24 h (BioRad; 120° angle, 60 to 240 s switch time, 4 V/cm). The gel was subsequently stained with ethidium bromide for analysis.

siRNA Treatment

Predesigned BRCA2 SMARTpool and scrambled siRNAs were purchased (Dharmacon, Lafayette, Colo.). 10000 cells seeded onto 6 well plates and left over night before transfected with 100 nM siRNA using Oligofectamine Reagent (Invitrogen) according to manufacturers instructions. Cells were then cultured in normal growth media for 48 h prior to trypsinisation and replating for toxicity assays. Suppression of BRCA2 was confirmed by Western blotting (as described previously [46]) of protein extracts treated with siRNA with an antibody against BRCA2 (Oncogene, Nottingham, UK).

EXAMPLES

Homologous Recombination Deficient Cells are Hypersensitive to PARP-1 Inhibition

To investigate the involvement of HR in cellular responses to inhibition of PARP-1, the effects of PARP-1 inhibitors on the survival of HR repair deficient cell lines were studied. It was found that cells deficient in HR (i.e., irs1SF which is defective in XRCC3 or irs1 which is defective in XRCC2 [see Table 1] were very sensitive to the toxic effect of 3-aminobenzamide (3-AB) and to two more potent inhibitors of PARP-1: 1,5-dihydroxyisoquinoline (ISQ; [37]) or 8-hydroxy-2-methylquinazolinone (NU1025 [38, 39]) (FIG. **1**). The sensitivity in irs1SF cells to 3-AB, ISQ or NU1025 was corrected by the introduction of a cosmid containing a functional XRCC3 gene (CXR3). Similarly, the sensitivity in irs1 cells to 3-AB, ISQ or NU1025 was corrected by the introduction of a cosmid containing a functional XRCC2 gene (irs1X2.2).

BRCA2 Deficient Cells are Hypersensitive to PARP-1 Inhibition

The survival of BRCA2 deficient cells (VC8) and wild type cells (V79Z) in the presence of inhibitors of PARP-1 was investigated. It was found that VC8 cells are very sensitive to the toxic effect of NU1025 (FIG. **2**). The sensitivity in VC8 cells was corrected by the introduction of a functional BRCA2 gene either on chromosome 13 (VC8#13) or on an overexpression vector (VC8+B2). This result demonstrates that the sensitivity to PARP-1 inhibitors is a direct consequence of loss of the BRCA2 function.

To investigate if inhibition of PARP-1 triggers apoptosis in BRCA2 deficient cells, the level of apoptosis 72 hours following exposure to NU1025 was investigated. It was found that NU1025 triggered apoptosis only in VC8 cells, showing that loss of PARP-1 activity in BRCA2 deficient cells triggers this means of death (FIG. **3**).

BRCA2 Deficient Breast Cancer Cells are Hypersensitive to PARP-1 Inhibition

It was examined whether the MCF7 (wild-type p53) and MDA-MB-231 (mutated p53) breast cancer cell lines displayed a similar sensitivity to NU1025 upon depletion of BRCA2. It was found that PARP inhibitors profoundly reduced the survival of MCF7 and MDA-MB-231 cells only when BRCA2 was depleted with a mixture of BRCA2 siRNA (FIG. **4**). This shows that BRCA2 depleted breast cancer cells are sensitive to PARP inhibitors regardless of p53 status.

BRCA2 Deficient Cells Die from PARP-1 Inhibition in Absence of DNA Double-Strand Breaks (DSBs) but in Presence of γH2Ax

HR is known to be involved in the repair of DSBs and other lesions that occur during DNA replication [2]. To determine whether the sensitivity of BRCA2 deficient cells is the result of an inability to repair DSBs following NU1025 treatment, the accumulation of DSBs in V79 and V-C8 cells was measured following treatments with highly toxic levels of NU1025. It was found that no DSBs were detectable by pulsed field gel electrophoretic analysis of DNA obtained from the treated cells (FIG. **5**A), suggesting that low levels of DSBs or other recombinogenic substrates accumulated following PARP inhibition in HR deficient cells, which trigger γH2Ax FIG. **5**B). The reason why BRCA2 deficient cells die following induction of these recombinogenic lesions is likely to be due to an inability to repair such lesions. To test this, the ability of BRCA2 deficient V-C8 cells and BRCA2 complimented cells to form RAD51 foci in response to NU1025 was determined. It was found that RAD51 foci were indeed induced in V-C8+B2 cells following treatment with NU1025 (statistically significant in t-test $p<0.05$; FIG. **5**D). This indicates that the recombinogenic lesions trigger HR repair in these cells allowing them to survive. In contrast, the BRCA2 deficient V-C8 cells were unable to form RAD51 foci in response to NU1025 treatment (FIG. **5**D) indicating no HR, which would leave the recombinogenic lesions unrepaired and thus cause cell death.

PARP-1 and Not PARP-2 is Important in Preventing Formation of a Recombinogenic Lesion

There are two major PARPs present in the nucleus in mammalian cells, PARP-1 and PARP-2 and all reported PARP inhibitors inhibit both. In order to distinguish which PARP was responsible for the effect, we tested if the absence of PARP-1 and/or PARP-2 results in accumulation of toxic lesions, by depleting these and BRCA2 with siRNA in human cells (FIG. **6***a*). We found that the clonogenic survival was significantly reduced when both PARP-1 and BRCA2 proteins were co-depleted from human cells (FIG. **6***b*). Depletion of PARP-2 with BRCA2 had no effect on the clonogenic survival and depletion of PARP-2 in PARP-1 and BRCA2 depleted cells did not result in additional toxicity. These results suggest that PARP-1 and not PARP-2 is responsible for reducing toxic recombinogenic lesions in human cells. The cloning efficiency was only reduced to 60% of control in PARP-1 and BRCA2 co-depleted cells, while no HR deficient cells survived treatments with PARP inhibitors. This is likely to do with incomplete depletion of the abundant PARP-1 protein by siRNA (FIG. **6***c*), which might be sufficient to maintain PARP-1 function in some of the cells.

PARP-1 is Activated by Replication Inhibitors

HR is also involved in repair of lesions occurring at stalled replication forks, which may not involve detectable DSBs [2]. To test if PARP has a role at replication forks, PARP activation in cells treated cells with agents (thymidine or hydroxyurea) that retard or arrest the progression of DNA replication forks was examined. Thymidine depletes cells of dCTP and slows replication forks without causing DSBs. Hydroxyurea depletes several dNTP and block the replication fork, which is associated with the formation of DSBs at replication forks [2]. Both of these agents potently induce HR [2]. V79 hamster cells treated for 24 hours with thymidine or hydroxyurea were stained for PAR polymers. This revealed a substantial increase in the number of cells containing sites of PARP activity (FIG. **7**C). This result suggests a function for PARP at stalled replication forks. It was also shown that inhibition of PARP with NU1025 enhances the sensitivity to thymidine or hydroxyurea in V-C8+B2 cells (FIG. **7**D,E). This result suggests that PARP activity is important in repair of stalled replication forks or alternatively that it prevents the induction of death in cells with stalled replication forks.

PARP is rapidly activated at DNA single-strand breaks (SSB) and attracts DNA repair enzymes [3-6]. Methylmethane sulphonate (MMS) causes alkylation of DNA, which is repaired by base excision repair. PARP is rapidly activated by the SSB-intermediate formed during this repair, which depletes the NAD(P)H levels (FIG. **7**F). We found that the activation of PARP and reduction of NAD(P)H levels is much slower following thymidine or hydroxyurea treatments. This slow PARP activation can be explained by the indirect action of thymidine and hydroxyurea and the time required to accumulate stalled replication forks as cells enter the S phase of the cell cycle.

PARP-1 and HR Have Separate Roles at Stalled Replication Forks

The number sites of PARP activity in untreated BRCA2 deficient V-C8 cells was determined. It was found that more V-C8 cells contain sites of PARP activity compared to V-C8+B2 cells (FIG. **8**A,B,C). Also, the V-C8 cells have lower free NAD(P)H levels than the corrected cells (FIG. **8**D), as a likely result of the increased PARP activity. Importantly these sites of PARP activity do not overlap with RAD51 foci (FIG. **8**E).

The results herein suggest that PARP and HR have separate roles in the protection or rescue of stalled replication forks (FIG. **8**F). A loss of PARP activity can be compensated by increased HR while a loss of HR can be compensated by increased PARP activity. However, loss of both these pathways leads to accumulation of stalled replication forks and to death, as in the case of PARP inhibited BRCA2 deficient cells.

As shown in the model outlined in FIG. **8**F PARP and HR have complementary roles at stalled replication forks. (i) Replication forks may stall when encountering a roadblock on the DNA template. In addition, they may also stall temporarily, due to lack of dNTPs or other replication co-factors. (ii) PARP binds stalled replication forks or other replication-associated damage, triggering PAR polymerization. Resulting negatively charged PAR polymers may protect stalled replication forks, by repelling proteins that normally would process replication forks (e.g., resolvases), until the replication fork can be restored spontaneously when dNTPs or other co-factors become available. Alternatively, PAR polymers or PARP may attract proteins to resolve the replication block by other means. (iii) In absence of PARP activity, HR may be used as an alternative pathway to repair stalled replication forks. This compensatory model explains the increased level of HR and RAD51 foci found in PARP deficient cells[3-5] and higher PARP activity found in HR deficient cells (i.e. V-C8). Spontaneous replication blocks/lesions are only lethal in the absence of both PARP and HR.

TABLE 1

Genotype and origin of cell lines used in this study.

Cell line	Genotype	Defect	Origin	Reference
AA8	Wt	Wt	CHO	[41]
irs1SF	XRCC3^{-}	XRCC3^{-}, deficient in HR	AA8	[41]
CXR3	XRCC3^{-} + hXRCC3	Wt	irs1SF	[41]
V79-4	Wt	Wt	V79	[40]
irs1	XRCC2^{-}	XRCC2^{-}, deficient in HR	V79-4	[40]
irs1X2.2	XRCC2^{-} + hXRCC2	Wt	irs1	[40]
V79-Z	Wt	Wt	V79	[42]
VC8	BRCA2^{-}	BRCA2^{-}, deficient in HR	V79-Z	[42]
VC8#13	BRCA2^{-} + hBRCA2	Wt	VC8	[42]
VC8 + B2	BRCA2^{-} + hBRCA2	Wt	VC8	[42]

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cttaaaataa aaaaaaaaaa aaaaaaaaaa	5490

The invention claimed is:

1. A method of treatment of cancer cells defective in homologous recombination (HR), the method comprising:

identifying a human patient with a familial predisposition to gene-linked hereditary cancer,

wherein said cancer comprises cancer cells defective in homologous recombination;

identifying a compound which inhibits PARP-1, and

administering to said human patient a therapeutically effective amount of said compound.

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